REMARKS

Amendments

In the interest of expediting the prosecution of this Application, Applicants amend the paragraph starting from page 5, line 36 of the specification as well as Claims 1, 3, 7, 8, 9, 14, and 16. Applicants amend Claims 1, 3, 7, 8, 9, 14, and 16 in order to further clarify the subject matter claimed. Applicants reserve the right to reintroduce the original claims in one or more continuation type applications.

The paragraph starting from page 5, line 36 is amended to include "an amino acid sequence of or" in the definition of "a first fusion partner". The omission of such an element is obviously a typographic error. Support for this amendment is found throughout the entire specification, for example, in page 27, lines 27-35, page 29, lines 5-25 (Example 4), and Figure 4.

Claims 1, 14, and 16 are amended to replace the term 'derived" with "obtained". Support for this amendment is found throughout the entire specification, for example, in page 13, lines 1-7 and page 27, lines 6-10.

Claim 3 is amended to delete the 3B9. Support for this amendment is found, for example, in page 32, lines 14-19.

Claims 7, 8, and 9 are amended to describe the amino acid sequences of the CDRs more clearly. Support for the amendments are found, for example, in page 11, lines 4-12 and lines 34-36.

Applicants respectfully contend that the amendments will place the case in condition for allowance. No new matter is added in any of the above amendments and the Examiner is respectfully requested to enter the amendments and reconsider the application.

Remarks

Claims 1-9 and 14-18 are pending in the present application.

1. Double Patenting Rejections.

The Examiner rejects Claims 1-9 and 17-18 under 35 U.S.C. § 112 under the

judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 7-18 and 28-29 and 34-35 of U.S. Patent No. 5,914,110.

Applicants will obviate this rejection by filing a terminal disclaimer if and when the claims are deemed allowable.

2. 35 U.S.C. § 112, first paragraph.

A. Claim 3

The Examiner rejects Claim 3 under 35 U.S.C. 112, first paragraph as containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention. In particular, the Examiner alleges that the specification lacks complete deposit information for the deposit of 3426A11C1B9. Applicants respectfully traverse the rejection.

Without acceding to the Examiner's allegation, Applicants amend Claim 3 by deleting the monoclonal antibody 3B9. Monoclonal antibody 6A1 is produced by the hybridoma 3426A11C1B9, which was deposited as an accession number 93100620. The proper and sufficient description of the deposit is found in page 32, lines 14-19. The rejection is therefore rendered moot.

For the reasons stated above, Applicants respectfully request that the Examiner withdraw the rejection of Claim 3 under 35 U.S.C. §112, first paragraph.

B. Claims 17-18

The Examiner rejects Claims 17-18 under 35 U.S.C. § 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention. Applicants respectfully traverse this rejection.

Antibody-based therapy methods for in vivo treatment of human disease are widely used.

The Examiner asserts that the claimed invention pertains to the highly experimental and unpredictable field of *in vivo* therapy using monoclonal antibodies, and

that the effective application of antibody-based therapy methods for *in vivo* treatment of human diseases has been extremely limited.

Applicants respectfully disagree with the Examiner's position. The law is clear that no working examples are required in a specification for it to meet the requirement of §112, first paragraph. *In re Borkowski*, 422 F.2d 904, 164 USPQ 642 (C.C.P.A. 1970).

Antibody-based drugs have been widely used in human disease treatment including the clinical application in conditions associated with excess IgE production. Clinical studies confirmed that it is operable to use antibodies to reduce the excessive IgE level in a human patient for treating the conditions related to the excessive IgE production, such as allergy. For example, Genentech disclosed positive results from a 536 patient phase-II/II trials of its recombinant humanized monoclonal antibody, rhuMAB-E25 (BioWorld.RTM. Today, Nov. 10, 1998, p. 1; a copy of this reference is enclosed in this response). The positive results from anti-IgE antibody trials suggest that therapeutic strategies aimed at IgE down-regulation is operable.

The fusion proteins of the present invention have in vivo utility.

The Examiner also states that Applicants only provided *in vitro* assay results, and assets that the *in vitro* assays cannot duplicate the complex conditions of in vivo therapy, and are not correlatable to the treatment of allergies and other conditions associated with excess IgE production.

Applicants respectfully disagree with the Examiner's position and submit that the method of using the fusion protein of Claim 1 for treating conditions associated with excessive production of IgE in human is operable. The *in vivo* effect of anti-IL4 antibodies of the present invention has been tested and confirmed. A journal article by Hart, et al. demonstrated that the anti-IL4 antibody /MoA 3B9/ effectively reduces the IgE level of the SCID mouse model (Hart, et al., Clin. Exp. Immunol. 130: 93-100 (2002), a copy of this reference is enclosed in this response).

In this model, SCID mice that had been administered immune cells from house dust mite-sensitive human donors were administered IL-4 causing a two-fold increase in IgE production. Treatment of mice with MoAb 3B9 at the time of restitution inhibited IgE production in a dose dependent manner (Fig. 5). The highest does of MoAb 3B9

rendered IgE levels undetectable at all time-points (page 97, column 2). In addition, toxicology studies in cynomolous monkeys confirmed that the anti-IL4 antibodies are well-tolerated, suggesting that the antibodies can be used safely in human patients.

The specification teaches how to produce and use fusion proteins having binding specificity for IL4 of Claim 1.

The Examiner asserts that the specification does not teach how to produce and use functional proteins having binding specificity for IL4 , which have the structural elements defined by Claim 1. The Examiner further states Claims 1-4 require that the claimed fusion protein be comprised of amino acid sequences from only a single CDR. The Examiner then concludes that it is unlikely that fusion proteins as defined by the claims which may contain less than the full complement of CDRs would possess the functional characteristics high affinity binding to and neutralize IL4.

Applicants respectfully submit that Claim 1 is directed to "the fusion protein having a binding specificity for human IL4..." Thus, any fusion proteins that do not have such a binding specificity would not be within the scope of Claim 1. In addition, the specification describes in detail the making of the fusion proteins falling within the scope of Claim 1, such as the humanized and chimeric antibodies of the present invention.

For the reasons stated above, Applicants respectfully request that the rejection of Claims 17-18 under 35 U.S.C. § 112, first paragraph be withdrawn.

C. Claims 1-9

The Examiner rejects Claims 1-9 under 35 U.S.C. § 112, first paragraph, alleging that the specification, while being enabling for fusion proteins containing the full complement of CDR's, does not reasonably provide enablement for fusion proteins containing only one CDR or CDR's in an unspecified order. Applicants respectfully traverse this rejection.

The definition on page 5 of the specification states, "such fusion proteins are engineered antibodies, e.g., chimeric antibodies, humanized antibodies, or antibody fragments..." This definition indicates that the fusion proteins of the present invention will comprise at least the full variable regions of an immunoglobulin heavy or light chain.

These antibodies would therefore, have a framework and full components of CDRs.

Additionally, Claims 1-9 recite that the claimed fusion protein comprises a first fusion partner. According to the specification, such a fusion partner is an amino acid sequence of a human framework, therefore, the fusion protein in Claim 1 indeed does have a human framework.

Finally, the specification provides clear guidance on how to make the fusion protein of Claims 1-9, such as how to connect the CDRs of a non-human monoclonal antibody to a first fusion partner (pages 17 to 20). Example 3 and 4 also describe in detail the design, production, and purification of the humanized anti-IL4 antibodies having binding specificity for human IL4. Therefore, one skilled in the art would be able to make and use the fusion protein of Claim 1 by following these detailed guidelines.

For the reasons stated above, Applicants respectfully request that the rejection of Claims 1-9 under 35 U.S.C. § 112, first paragraph be withdrawn.

3. 35 U.S.C. § 112, second paragraph

The Examiner rejects Claims 1-9 and 14-18 under 35 U.S.C. § 112, second paragraph, as allegedly being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Applicants respectfully traverse the rejection.

a) The Examiner alleges that the terminology "is derived from" renders the claim vague and indefinite

Without acceding to the Examiner's allegation, Applicants have amended Claims 1, 14, and 16, and the term "is derived from" has been changed to "is obtained from", which has a clear and definite meaning to the skilled artisan.

b) The Examiner asserts that the terminology "neutralizing" renders the claim vague and indefinite, allegedly because is not clear which activity of IL-4 is neutralized.

However, page 15, lines 2-3 of the specification state, "the engineered antibodies of the invention are neutralizing, i.e., they desirably block binding to the receptor of the IL4 protein." Therefore, it is clear which activity of IL-4 is neutralized.

c) The Examiner alleges that the terminology "a first fusion partner" renders the

claims vague and indefinite.

Without acceding to the Examiner's allegation, the definition of the "first fusion partner" on page 5, line 36 of the specification has been amended to "an amino acid sequence of or a nucleic acid sequence encoding...."

d) The Examiner asserts that Claim 2 is vague and indefinite, allegedly because it is not clear how and where the second fusion partner is attached to the fusion protein.

Page 6, lines 14-29 of the specification clearly explain where and how the second fusion partner is attached to the fusion protein. The second fusion partner is fused in frame or by means of an optional conventional linker sequence (e.g. operatively linked). In addition, Example 3 (page 27, line 28-30) also describes how and where a second fusion partner (IgG1 human constant region) is connected to the variable region of the humanized antibody, specifically that the IgG1 human constant region is connected in frame to the C-terminus of the humanized heavy chain variable region. Standard molecular cloning techniques are used to achieve such connection.

e) The Examiner asserts that Claim 4 is indefinite allegedly because it is not known which particular part of heavy chain or light chain the claim refers to.

Applicants respectfully submit that Claim 4 refers to the portion of heavy or light chain constant region. Therefore, the Examiner incorrectly alleges that the portion may be part of the variable region. In addition, Applicants believe that the meaning of "part of an immunoglobulin constant heavy chain or immunoglobulin constant light chain" is clear and it would refers to any portion of the immunoglobulin constant heavy or light chain.

f) The Examiner asserts that Claims 5 and 6 are vague and indefinite allegedly because it is not clear what and how the recited sequences are linked.

The recited sequences are the framework sequences. It should be clear to the one skilled in the art that the framework sequences are linked to the sequences of the CDRs. Example 3 describes in detail how the recited sequences of Claims 5 and 6 are linked to the CDRs.

g) The Examiner alleges that there is no antecedent basis for "said fusion partner sequence"

Applicants respectfully point out that the language in Claims 5 and 6 is "said first fusion partner sequence" instead of "said fusion partner sequence". Claim 1 recites "a first fusion partner" and thereby provides the antecedent basis for the phase "said first fusion partner sequence" in Claim 6.

h) The Examiner alleges that there is no antecedent basis for "said amino acid sequences" in Claims 7-9.

Claims 7-9 have been amended to recite "The fusion protein according to claim 1, wherein the complementarity determining regions for the ... chain comprise ...". The terms in the amended Claims 7-9 have antecedent basis and the rejection is thereby rendered moot.

i) The Examiner alleges that the first amino acid sequence in SEQ ID NO. 16 of Claim 8 should be lys instead of leu.

changes not made

Applicants have amended Claim 8 by changing leu to lys. Applicants appreciate the Examiner's careful review of the claims.

j) The Examiner alleges that in Claims 7-9, it is unclear if each amino acid sequence is the CDR or part of the CDR, and it is also unclear if each sequence can be present three times to give the full complement of CDR's or each only present once.

Applicants have amended Claims 7-9. The amended Claims 7-9 now indicate the information as to the amino acid sequences of the CDRs.

In view of the foregoing arguments and amendments, Applicants believe that the claims now comply with the requirement of 35 U.S.C. § 112, second paragraph. Accordingly, Applicants respectfully request that the rejection be withdrawn.

4. 35 U.S.C. § 102(a)

A. WO93/04173

The Examiner rejects Claims 1, 8-9, and 17 as allegedly being anticipated by WO 93/04173. Applicants respectfully traverse this rejection.

WO 93/04173 does not teach a fusion protein of Claim 8.

For a prior art reference to anticipate, every element of the claimed invention must be identically shown in a single reference. *Diversitech Corp. v. Century Steps, Inc.*, 850

F.2d 675, 677, 7 U.S.P.Q.2d 1315, 1317 (Fed. Cir. 1988).

WO 93/04173 discloses Mae15 (an anti-IgE antibody), whose light chain contains the sequence AASNLES. The amended Claim 8 recites the light chain CDRs comprising a first CDR of SEQ ID NO: 16, a second CDR of SEQ ID NO 18, and a third CDR of SEQ ID NO: 28. WO 93/04173 does not teach a fusion protein having such CDRs and therefore it does not anticipate Claim 8. Since Mae15 is an anti-IgE antibody and does not have the identical amino acid sequence of the fusion protein of Claim 8, Mae15 would not have the ability to neutralize IL4 with the claimed dissociation constant of the present invention.

WO 93/04173 does not teach each and every element of Claims 1, 8-9, and 17.

Claim 1 is directed to a fusion protein having binding specificity for human interleukin-4 (IL4) which comprises CDRs derived from a non-human neutralizing monoclonal antibody characterized by a dissociation constant equal to or less than 2×10^{-10} M for human IL4, and a first fusion partner.

WO 93/04173 merely discloses anti-IgE antibodies, which are different from the fusion protein having binding specificity for IL4 as in Claim 1. The former binds to IgE while the latter has binding specificity for IL4.

Furthermore, WO 93/04173 does not disclose a monoclonal antibody having a dissociated constant equal to or less than 2×10^{-10} M or any fusion protein derived from such monoclonal antibodies. In fact, the antibodies disclosed in WO 93/04173 all have an affinity constant with IgE of more than 10^{-9}M (see Table 5a).

Accordingly, for the reasons stated above, Applicants respectfully submit and request that the Examiner withdraw the rejection under 35 U.S.C. § 102(a).

B. WO 93/17106

The Examiner rejects Claims 1-4, 14-17 as being anticipated by WO 93/17106. Applicants respectfully traverse this rejection.

WO 93/17106 does not disclose any fusion proteins, or humanized antibodies or chimeric antibodies that are derived from a monoclonal antibody having a dissociation constant equal to or less than 2×10^{-10} M as recited in Claims 1, 14, and 16. The antibody 25D2 has an affinity constant for IL4 of 1×10^{-9} M (page 66, Table 5), which is more than

2×10^{-10} M. Because WO 93/17106 does not disclose each and every element of the claims, Applicants respectfully request that the rejection of Claims 1-4, 14-17 under 35 U.S.C. § 102(a) over WO 93/17106 be withdrawn.

5. 35 U.S. C. § 102(b)

A. EP327000

The Examiner rejects Claims 1-2, 4, 8-9, 14 and 16-17 as being anticipated by EP327000. Applicants respectfully traverse this rejection.

EP327000 does not teach an antibody or a fusion protein that inherently has the ability to neutralize IL4 with the claimed dissociation constant of the present invention.

EP327000 discloses anti-HIV chimeric antibodies wherein the light chain contains the sequence that is the same as SEQ ID NO: 16 of the present invention. As presently amended Claim 8 recites the light chain CDRs comprising a first CDR of SEQ ID NO: 16, a second CDR of SEQ ID NO 18, and a third CDR of SEQ ID NO: 28. EP327000 does not teach an antibody or fusion protein having such CDRs and therefore it cannot anticipate Claim 8. Since the antibodies in EP327000 do not have the identical amino acid sequence of the fusion protein of Claim 8, the antibodies of EP327000 will only bind to HIV and will not bind to human IL4 or inherently have the ability to neutralize IL4 with the claimed dissociation constant of the present invention.

Furthermore, the antibodies of EP32700 bind to HIV virus instead of IL4. Therefore, EP327000 does not teach two important elements of Claim 1, 14 and 16: a) the binding specificity for human IL4, and b) "a dissociation constant equal to or less than about 2×10^{-10} M for human IL4". As a result, this reference cannot anticipate Claims 1-2, 4, 8-9, 14 and 16-17.

For the reasons stated above, Applicants respectfully request that the rejection of Claims 1-2, 4, 8-9, 14 and 16-17 under 35 U.S.C. § 102(b) over EP327000 be withdrawn.

B. Perfetti et al. Mole. Immunol. Vol. 287 p. 505 (1991).

The Examiner rejects Claims 1-4 and 7 as being anticipated by Perfetti et al. Mole. Immunol. Vol. 287 p. 505 (1991). Applicants respectfully traverse this rejection.

The amended Claim 7 have the heavy chain CDRs comprising a first CDR of SEQ

ID NO: 22, a second CDR of SEQ ID NO: 24, and a third CDR of SEQ ID NO: 26. Perfetti et al. disclose an isogeneic monoclonal antiidiotype to an anti- α DEXTRAN antibody having the heavy chain containing thr-ser-gly-met-val-ser (SEQ ID NO: 22 of the present invention) and the production of said heavy chain using recombinant technology. Since Perfetti et al do not disclose a fusion antibody having the identical or even substantially similar amino acid sequence of the fusion protein of Claim 7, the antibodies of Perfetti et al would only bind to α DEXTRAN antibody and would not bind to human IL4, or have the ability to neutralize IL4 with the claimed dissociation constant of the present invention.

For the reasons stated above, Applicants respectfully request that the rejection of Claims 1-4 and 7 under 35 U.S.C. § 102(b) over Perfetti et al. be withdrawn.

C. EP00327283

The Examiner rejects Claims 1 and 18 as being anticipated by EP00327283. The rejection is respectfully traversed.

EP00327283 discloses the use of monoclonal antibodies against human IL-4 in treating allergies. However, it does not disclose a fusion protein derived from a monoclonal antibody characterized by a dissociation constant equal to or less than 2×10^{-10} M for human IL4. Thereby, this reference does not teach each and every element of Claims 1 and 18 and does not anticipate Claims 1 and 18.

For the reasons stated above, Applicants respectfully request that the rejection of Claims 1 and 18 under 35 U.S.C. § 102(b) over EP00327283 be withdrawn.

6. 35 U.S.C. § 102(b)/103(a)

The Examiner rejects Claim 1 under 35 U.S.C. § 102(b) as anticipated by or, in the alternative under 35 U.S.C. § 103(a), as obvious over Ramanathan et al. WO 91/09059 or JP-327725 or Chreiten et al. J. Immunol. Methods vol. 117 p. 67 (1991). The Examiner alleges that these three references disclose the neutralizing monoclonal antibodies against human IL-4. The Examiner also asserts that the claimed fusion protein is deemed to be the same as disclosed in the cited references. The Examiner further

concludes that a dissociation constant of less than 2 x 10^{-10} M is deemed to be an inherent characteristic of the referenced antibodies since most monoclonal antibodies have affinity constants of 2 x 10^{-10}M or less. Applicants respectfully traverse this rejection.

A. Novelty

WO 91/09059 discloses mouse monoclonal antibody produced by immunization with a peptide corresponding to residue 61-82 of human IL-4. JP-327725 discloses mouse monoclonal antibody specific for human IL-4 which neutralize IL-4 activity. Cretien et al., disclose rat monoclonal antibody 11B4 which inhibits the TCGF bioactivity of human IL-4. Claim 1 of the present invention, on the other hand, recites a fusion protein having binding specificity for human interleukin-4 (IL4) which comprises complementarity determining regions (CDRs) derived from a non-human neutralizing monoclonal antibody characterized by a dissociation constant equal to or less than 2 x 10^{-10} M for human IL4, and a first fusion partner. Accordingly, the present fusion protein is novel over the cited references.

*The cited references do not anticipate the **fusion** protein of Claim 1.*

The antibodies disclosed in the cited references are produced by hybridoma methodology. These antibodies are different from the present fusion protein. As stated in the specification (page 5, line 26-30), "fusion protein" refers to "a protein encoded by a fusion molecule, which may be obtained by expression in a selected host cell. Such fusion proteins are engineered antibodies, which are further defined as a type of fusion protein, i.e., a synthetic antibody in which a portion of the light and/or heavy chain variable domains of a selected acceptor antibody are replaced by analogous parts from one or more donor antibodies..." Thus, a fusion protein of the present invention comprises portions that are obtained from separate antibodies. The antibodies in the cited references are not fusion proteins. On the contrary, they are the homogenous antibodies produced by the natural immune system of a host animal.

*The cited references do not anticipate the **fusion** protein derived from a monoclonal antibody characterized by **a dissociation constants equal to or less than 2 x 10^{-10} M** for human IL4 of Claim 1.*

As a preliminary matter, Applicants respectfully contend the validity of the Examiner's assertion that "most monoclonal antibodies have [dissociation] constants of 1 x 10^{-10}M or less". The Examiner does not provide any evidence to support the allegation that "most monoclonal antibodies have constants of 1 x 10^{-10}M or less". The court in *In re Zurko* (59 USPQ2d 1693, 1697 (Fed. Cir.)) held that "With respect to core factual findings in a determination of patentability...the Board cannot simply reach the conclusion based on its own understanding or experience—or on its assessment of what would be basic knowledge or common sense. Rather, the Board must point to some concrete evidence in the record in support of these findings.". In the present application, Applicants respectfully point out that the Examiner has not pointed to any concrete evidence in support of the allegation that "most monoclonal antibodies have [dissociation] constants of 1 x 10^{-10}M or less."

In addition, the cited references do not disclose or suggest any antibodies that having such dissociation constants. Indeed, the experimental data in one of the cited references in this office action, WO 93/04173, teaches quite the opposite. In that reference, the dissociation constants of at least 7 monoclonal antibodies were tested and disclosed (Table 5a). None of the monoclonal antibodies have the dissociation constant less than 2 x 10^{-10} M or 1 x 10^{-10} M.

B. Obviousness

Under U.S. patent law, to establish a *prima facie* case of obviousness under 35 U.S.C. §103, three basic criteria must be met. First, there must be some suggestion or motivation, either in the reference itself, or in the knowledge generally available to one of the skilled in the art, or modify the reference or to combine reference teachings. *In re Fine*, 837 F.2d 1071, 5 USPQ2d 1596 (Fed. Cir. 1988) Second, there must be a reasonable expectation of success. *In re Merck & Co. Inc.*, 800 F.2d 1091, 231 USPQ 375 (Fed. Cir. 1986) Finally, the prior art reference must teach or suggest all the claim limitations. *In re Royka,* 490 F.2d 981, 180 USPQ 580 (CCPA 1974).

The Examiner fails to establish a prima facie case of obviouness.

Applicants again respectfully submit that the Examiner has not pointed to any

concrete evidence in record in support of the Examiner's allegation that "most monoclonal antibodies have constants of 1 x 10^{-10}M or less.", as required by the holding in *In re Zurko* (59 USPQ2d 1693, 1697 (Fed. Cir.)), therefore the Examiner fails to establish a *prima facie* case of obviousness.

*There is no reasonable expectation of success to make a **fusion** protein of Claim 1 in view of the cited references.*

The cited references do not disclose any guidance as to how to make the fusion protein of Claim 1. The cited references only discuss the making of monoclonal anti-IL4 antibodies with hybridoma techniques, which is entirely different from the recombinant approaches of the present invention. As disclosed in the present invention, making the fusion protein of Claim 1 requires complicated steps involving both molecular cloning and protein engineering, which have been described in the present invention in a clear fashion. However, the cited references do not even mention a general guideline as to the making of a fusion protein. Even assuming, *arguendo*, that the cited references suggested the making of the fusion protein as presently claimed, there would be no reasonable expectation, *a priori,* that such a fusion protein would exhibit the presently-claimed dissociation constant.

For the reason stated above, Applicants respectfully request that the 35 U.S.C. §§ 102(b)/103(a) rejection of Claim 1 over WO 91/09059 or JP-327725 or Chreiten et al. (J. Immunol. Methods vol. 117 p. 67 (1991)) be withdrawn.

7. 35 U.S.C. § 103(a)

The Examiner rejects Claims 1-4 and 14-17 under 35 U.S.C. § 103(a) as being unpatentable over Queen et al. WO 90/07861 in view of Abrams et al. U.S. 5,041,381, Chreiten et al J. Immunol. Methods Vol. 117 p.67 (1991) and Curtis et al. U.S. 5,108,910. Applicants respectively traverse the rejection.

The Examiner asserts that WO 90/07861 teaches methods for producing fusion proteins, which are chimeric or CDR grafted humanized antibodies. The Examiner further asserts that U.S. 5,041,381 teaches rat monoclonal antibody 1C1.11B 4.6 which has specificity for human IL-4, and 5,108,910 teaches the advantages of an amino acid

sequence of the fusion protein being linked to an additional peptide. The Examiner further asserts that a large proportion of the antibodies would have been expected to have the dissociated constants of 1×10^{-10} or less. The Examiner then concludes that it would be *prima facie* obvious to one of ordinary skill in the art at the time of the invention to apply the methods taught by Queen et al in order to develop the fusion protein. Applicants respectfully traverse this rejection.

The Examiner fails to establish a prima facie case of obviousness.

Again, as a preliminary matter, Applicants respectfully contend the validity of the Examiner's assertion that "a large proportion of the antibodies would have been expected to have dissociated constants of 1×10^{-10} M or less". Applicants respectfully submit that the Examiner has not put forth any concrete evidence in support of the allegation that "a large proportion of the antibodies would have been expected to have dissociation constants of 1×10^{-10} or less. Therefore the Examiner fails to establish a *prima facie* case of obviousness.

There is no reasonable expectation of success in combining WO 90/07861 and U.S. 5,041,381, Chreiten et al and U.S. 5,108,910.

There is no reasonable expectation of success in combining the cited references.

WO 90/07861 discloses the making of the humanized anti-IL2 antibody. The guideline set forth in WO 90/07861 requires the analysis of the amino acid sequence of a donor immunoglobulin. For instance, Criterion I of the guideline states: "as acceptor, use a framework from a particular human immunoglobulin that is unusually homologous to the donor immunoglobulin to be humanized" (page 12, lines 17-20). In order to perform this step, the amino acid sequence of the donor imunoglobulin must be compared to the framework of a human acceptor immunoglobulin. In other words, if one skilled in the art is going to make a humanized anti-IL4 antibody, he or she must know the amino acid sequence of the donor anti-IL4 antibody. None of the above-cited references disclose the amino acid or even polynucleotide sequences of anti-IL4 antibodies. Accordingly, one skilled in the art would not be able to humanize anti-IL4 antibodies by following the guideline in WO 90/07861 based on the information provided in U.S. 5,041,381, Chreiten et al and U.S. 5,108,910, alone or in combination.

For the reasons stated above, Applicants respectfully request that the Examiner's rejection of Claims 1-4 and 14-17 under 35 U.S.C. § 103(a) over WO 90/07861 in view of US 5041381, Chreiten et al and U.S. Pat. No. 5,108,910 be withdrawn.

CONCLUSION

In view of the foregoing amendment and remarks, the Applicants believe that the application is in good and proper condition for allowance. If, in the opinion of the Examiner, a telephone conference would expedite the prosecution of the subject application, the Examiner is invited to call the undersigned at (650) 463-8109.

Respectfully submitted,

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Enclosures: BioWorld.RTM.Today, Nov. 10, 1998, p.1; and
Hart, et al., Clin. Exp. Immunol. 130:93-100 (2002)

MARKED-UP VERSION INDICATING CHANGES MADE

In the Specification

Please amend the paragraph starting from page 5, line 36 of the specification as follows:

"First fusion partner" refers to an amino acid sequence of or a nucleic acid sequence encoding a human framework or human immunoglobulin variable region in which the native (or naturally-occuring) CDRs are replaced by the CDRs of a donor antibody. The human variable region can be an immunoglobulin heavy chain, a light chain (or both chains), an analog or functional fragments thereof. Such CDRs or CDR regions, located within the variable region of antibodies (immunoglobulins) can be determined by known methods in the art. For example, Kabat et al., [Sequences of Proteins of Immunological Interest, 4th Ed., U.S. Department of Health and Human Services, National Institutes of Health (1987)], disclose rules for locating CDRs. In addition, computer programs are known which are useful for identifying CDR regions/structures.

In the Claims

Please amend Claims 1, 3, 7, 8, 9, 14, and 16 as follows:

1. (Amended) A fusion protein having binding specificity for human interleukin-4 (IL4) which comprises complementarity determining regions (CDRs) ~~derived~~ obtained from a non-human neutralizing monoclonal antibody characterized by a dissociation constant equal to or less than 2×10^{-10} M for human IL4, and a first fusion partner.

3. (Amended) The fusion protein according to claim 1 wherein said non-human neutralizing monoclonal antibody is ~~selected from the group considered of 3B9 and~~ 6A1.

7. (Amended) The fusion protein according to claim 1 wherein ~~said amino acid sequences of~~ the complementarity determining regions for the heavy chain ~~are~~comprise:

(a) ThrSerGlyMetGlyValSer: SEQ ID NO: 22,

(b) HisIleTyrTrpAspAspAspLysArgTyrAsnProSerLeuLysSer: SEQ ID NO: 24, ~~or~~and

(c) ArgGluThrValPheTyrTrpPheAspVal: SEQ ID NO: 26.

8. (Amended) The fusion protein according to claim 1 wherein ~~said amino acid sequences of~~ the complementarity determining regions (CDRs) for the light chain ~~are~~comprise:

(a) LeuAlaSerGlnSerValAspTyrAspGlyAspSerTyrMetAsn: SEQ ID NO: 16,

(b) AlaAlaSerAsnLeuGluSer: SEQ ID NO: 18, and~~or~~

(c) GlnGlnSerAsnGluAspProProArg: SEQ ID NO: 28.

9. (Amended) The fusion protein according to claim 1 wherein ~~said amino acid sequences of~~ the complimentarity determining regions (CDRs) for the light chain ~~are~~comprise:

(a) LysAlaSerGlnSerValAspTyrAspGlyAspSerTyrMetAsn: SEQ ID NO: 16,

(b) AlaAlaSerAsnLeuGluSer: SEQ ID NO: 18, ~~or~~and

(c) GlnGlnSerAsnGluAspProProThr: SEQ ID NO: 20.

14. (Amended) A humanized antibody comprising a heavy chain and a light chain, said antibody characterized by a dissociation constant equal to or less than about 2×10^{-10} M for human IL4, wherein the framework regions of said heavy and light chains are ~~derived~~ obatined from at least one selected human antibody and the amino acid sequences of the complementarity determining regions of said chain are ~~derived~~ obtained from a non-human neutralizing monoclonal antibody specific for human IL4 characterized by a dissociation constant equal to or less than about 1×10^{-10} M for human IL4.

16. (Amended) A chimeric antibody comprising a heavy chain and a light chain, said antibody characterized by a dissociation constant equal to or less than about 2×10^{-10} M for human IL4, wherein the amino acid sequences of the complimentarity determining regions of said heavy chain and said light chain are ~~derived~~ obtained from a non-human neutralizing monoclonal antibody specific for human IL4 characterized by a dissociation constant equal to or less than about 2×10^{-10} M for human IL4.

89-229288/32

B0098

chloro-23(E)-methoximino Factor A (IIa).

A soln. of (IIa) (87 mg.) and 2,2'-3-bis-(2-methylproprionitrile) (3 mg.) in refluxing dry toluene (3 ml.) was treated, under N_2, with tri-n-butyl tin hydride (192 mg.). After 15 min. the soln. was cooled and evapd.

The residual oil was dissolved in hexane/EtOAc (4 : 1). The soln. was filtered through Kiselgel 60. The filtrate was evapd. HPLC purifcn. of the residual foam gave 10 mg. 5-desoxy-23-methoximino Factor A, $[\alpha]_D^{21}$ +141° (c 0.3, $CHCl_3$). (20pp985JMDwgNo0/0).

(E) ISR: No Search Report.

EP-327270-A/2

89-229298/32 B03 C02 **SANY 29.01.88**

SANKYO KK *EP -327-280-A

22.07.88-JP-183127 (+JP-019125) *(09.08.89)* A61k-37/02 A61k-47

Pharmaceutical compsn. - comprises at least one cyclosporin in admixture with at least one mono-or-di:glyceride of fatty acid to dissolve cyclosporin

C89-101740 R(AT BE CH DE ES FR GB GR IT LI LU NL SE)

Other Priority: 10.05.88-JP-111639

Pharmaceutical compsn. comprises at least one cyclosporin in admixture with an amt. of at least one mono- or di-glyceride of a 6-10C fatty acid sufficient to dissolve the cyclosporin.

USE/ADVANTAGE

The compsn. is formulated for oral admin. or for the admin. to the eyes. The compsn. is useful for suppressing the mammalian immune system and for treating the ocular symptoms of Behcet's syndrome. It may also be useful for treating keratoplasty, herpetic keratitis and spring catarrh.

The cyclosporins have excellent solubility in mono- and di- glycerides of intermediate mol.wt. fatty acids which are easily emulsified in water and this improves the dispersibility of cyclosporin in water and aq. media.

BC(2-C, 10-E4C, 10-E4D, 12-D2B, 12-L4)

5

B0099

PREFERRED COMPOSITION

The fatty acid is caproic acid, 4-methylpentanoic acid, enanthic acid, 5-methylhexanoic acid, 2-ethylhexanoic acid, caprylic acid, 6-methylheptanoi acid, pelargonic acid or 8-methylnonanoic acid. The wt. ratio of the glyceride to the cyclosporin is 1:0.25 to 1:0.5 and the concn. of cyclosporin is 0.2-200 mg/ml. The compsn. is in the form of an oily soln. or aq. emulsion contg. non-aq. components are in amts. of ≤ 25% of the compsn.

EXAMPLE

Cyclosporin (1 part), caprylic acid diglyceride (2 parts), pluronic F68 (4 parts) and physiological saline (96 parts) were emulsified. No separation of cyclosporin could be observed.

The emulsion brought about good absorbability compared with prior art compsns. (12pp1917JMDwgNo0/0).

(E) ISR: EP-170623 EP-143305 GB2015339.

EP-327280-A

89-229300/32 B04 D16 **SCHE 02.02.88**

SCHERING BIOTECH CO *EP -327-283-A

02.02.88-US-151413 *(09.08.89)* A61k-39/39 C12p-21

Reducing inappropriate IgE responses, e.g. for treating allergy - by administration of antagonist to interleukin-4, pref. monoclonal antibody fragment of binding compsn.

C89-101741 R(ES GR)

Method of reducing an immunoglobulin E (IgE) response comprises admin. of an effective amt. of an antagonist to human IL-4 (interleukin-4).

USE

For reducing inappropriate IgE responses in allergic diseases and immune disorders associated with excessive IgE prodn.

IL-4 ANTAGONIST

Prefd. IL-4 antagonists are monoclonal antibodies, their fragments or binding compsns. comprising heavy and light chain variable regions, capable of blocking the IgE enhancing activity of human IL-4. The fragment is pref. an Fab fragment.

Doses are pref. 1-20mg/kg, i.v., pref. at a concn. of 1-20mg/ml.

B(4-B4C5, 12-D2) D(5-H11)

21

B0100

The monoclonal antibodies are produced conventionally, pref. from human-human hybridomas produced by fusing peripheral blood lymphocytes with a myeloma cell line.

EXAMPLE

None given. (6pp1762DAHDwgNo0/0).

(E) ISR: EP-255547 EP-245767.3.Jnl.Ref.

EP-327283-A

93-288412/36 B04 D16 **SCHE 92.02.19**
SCHERING CORP *WO 9317106-A1
92.02.19 92US-841659 *(93.09.02)* C12N 15/13, A61K 39/395, C07K 15/00, C12P 21/08, C12N 5/10, 5/20

Monoclonal antibodies against human interleukin-4,corresp. DNA and CDRs - are useful for detection of interleukin-4 and treatment of related diseases (Eng)

C93-128766 N(AU BB BG BR CA CZ FI HU JP KR LK MG MN MW NO NZ PL RO RU SD SK UA US) R(AT BE CH DE DK ES FR GB GR IE IT LU MC NL OA PT SE)

A monoclonal antibody (MAb1), produced by hybridoma ATCC HB 9809, is new.

Also claimed are :-

(a) hybridoma ATCC HB 9809;

(b) a polypeptide comprising a variable (V) region of anti-human interleukin-4 (hIL4) monoclonal antibody (Ab) 25D2 (sequence given in specification) or a subsequence of this;

(c) isolated DNA with 12-363 or 9-321 bases of DNA encoding the heavy and light chain V regions, respectively, of 25D2 (DNA sequences are given in the specification);

(d) a chimeric or humanised Ab specifically binding human IL4 and comprising complementarity determining regions (CDRs) from the heavy and light chain V regior of MAb1;

(e) DNA encoding a heavy or light chain V region i (d);

(f) a recombinant vector contg. the DNA of (c) or and

(g) a host cell contg. (f).

B(4-B4A1, 4-B4A3, 4-B4C5, 4-B4C6, 4-C1) D(5-H3B, 5-H8, 5-H11, 5-H12) B04

5

USE/ADVANTAGE

MAb1, the Ab of (d) and binding compsns. specific human IL-4 comprising V regions/CDRs from MAb1 are hIL-4 antagonists. Pharmaceutical compsns. contg. thei may be used for detecting, measuring and immunopur.., human IL4 and for blocking IL4 activity in IL4-related diseases.

The humanised Abs have reduced immunogenicity.

PREFERRED ANTIBODY

The humanised monoclonal Ab pref. comprises heavy and/or light chain V regions with the amino acid sequer of h25D2L-1, h25D2H-1, h25D2H-2, h25D2H-3, h25D2H-4 h25D2H-5.

WO9317106-

EXAMPLE

A male Lewis rat was immunised intraperiton ally (i.p.) with 1 ml human IL4 soln. emulsified with 1 ml complete Freund's adjuvant (CFA). The IL4 soln. consisted of human IL4 at 14 µg/ml in 10 mM Tris-HCl, 0.5 M NaCl, pH 7.4. The human IL4 was produced recombinantly from COS-7 host cells (ATCC CRL 1651) transfected with the expression vector pcD-hIL4 (ATCC 67029), and hIL4 had a specific activity of 2×10^7 U/mg.

Two wks. after the initial immunisation, the rat was injected i.p. with 1 ml hIL4 soln. emulsified with 1 ml CFA. Three months after the second injection, the rat was boosted intravenously with 1 ml hIL4 soln. (15 µg). Four days later, the rat was killed, blood was collected and the spleen removed for fusion.

Spleen cells were fused with mouse myeloma cells P3X63-Ag8.653 (ATCC CRL 1580), in a 1:1 ratio using polyethylene glycol. The cell suspension (3.5×10^5 cells in HAT medium) was put into 40 x 96-well plates. Ten days later, hybridoma supernatants were tested for their ability to bind hIL4 immobilised directly on microtitre plates (indirect ELISA) or to hIL4 bound to immobilised polyclonal IgG fraction of rabbit anti-hIL4.

Hybridomas secreting Abs reacting with IL4 were cloned by limiting dilution. The hybridomas were screened further for ability of their Abs to block the TCGF activity of hIL4. MAbs produced by hybridoma MP4.25D2.11 had t highest titre of blocking activity and were of rat IgG1 t Hybridoma MP4.25D2.11 was deposited as ATCC HB 980 (114pp2334CAFDwgNo0/6).

SR:3.Jnl.Ref EP314402

Addnl. Data: ABRAMS J S, DALIE B, LE H V, MILLER K, MURGOLO NGUYEN H, PEARCE M, TINDALL S, ZAVODNY P J 93.02.18 93WO-US01301

WO9317106-

93-288413/36 B04 D16 **MEDI- 92.02.22**
MEDICAL RES COUNCIL *WO 9317107-A1
92.02.22 92GB-003821 *(93.09.02)* C12N 15/30, A61K 39/015, C07K 7/10, C12N 1/21 // C12N 15/62

Allelic variants of epidermal growth factor 1- or 2-like domains - of merozoite surface protein 1, produced recombinantly for malaria vaccines (Eng)

C93-128767N(AT AU BB BG BR CA CH CZ DE DK ES FI GB HU JP KP KR LK LU MG MN MW NL NO NZ PL PT RO RU SD SE SK UA US) R(AT BE CH DE DK ES FR GB GR IE IT LU MC NL OA PT SE)

A polypeptide (I) is new, comprising one of two allelic variants of the epidermal growth factor (EGF)-1- or -2-like domain (given in the specification), in isolation from naturally occurring adjacent sequences in the merozoite surface protein-1 (MSP1).

Also claimed are:

(a) a nucleotide sequence encoding (I);

(b) a vector contg. (a);

(c) a host cell contg. (b); and

(d) a vaccine comprising a polypeptide (I) and a physiologically acceptable carrier.

USE/ADVANTAGE

(I) may be used alone or as a fusion protein of EGF-1-like and EGF-2-like domains in vaccines against malaria. (I expressed recombinantly is produced in a form indisting able from that in the native protein.

B(2-V2, 4-B2B1, 4-B4A1, 4-C1) D(5-C11, 5-H3B, 5-H7, 5-H12) B0

4

PREFERRED POLYPEPTIDE

The vector expresses (I) in the conformation adopt in MSP1. (I) may be expressed as a fusion protein, p contg. a moiety that facilitates purificn. by, e.g. enab (I) to be cleaved from the fusion protein.

EXAMPLE

DNA encoding the combined EGF-like domains of Plasmodium yoelii MSP1 was synthesised by PCR amplifi tion of P. yoelii genomic DNA using primers (given in specification). The amplification prods. were treated v BamHI and BglII, purified, then ligated into the expre vector pGex3x.

After transforming Escherichia coli DH5α cells to ar cillin resistance, individual clones were screened by di tion and protein prodn. A single clone expressing the appropriate fusion protein (i.e. the two EGF-like dom fused to the C-terminus of glutathione S-transferase (were selected and the DNA sequence determined.

WO9317107-

SLOAN-KETTERING INST *WO 9109-058-A
14.12.89-US-450918 (27.06.91) A61k-39/39 A61k-43 C07k-15 C12n-15/09 G01n-33/53

r-variable region of monoclonal antibody M195 - used for ....t and diagnosis of leukaemia also for delivering genetic formation to a targetted cell

C91-090255 R(AT BE CH DE DK ES FR GB GR IT LU NL SE) N(AU CA JP US)

A polypeptide (A), having an amino acid sequence capable of binding to the antigen which monoclonal antibody (MAb) M195 (ATCC HB10306) binds to, is new.

Also claimed are:
(1) a chimeric Ab comprising (A); and
(2) a therapeutic agent comprising (A) conjugated to a cytotoxic agent.

USES

(A), or the therapeutic agent contg. (A), is useful in a pharmaceutical compsn. (claimed) for treating leukaemia. The therapeutic agent is present in the compsn. in an amt. of 0.05-500 mg., for intravenous administration to destroy leukaemic cells (pref. 0.01-50 mg).

A patient's natural bone marrow cells can be destroyed using the chimeric Ab or the therapeutic agent.

D(5-H9, 5-H11)

Leukaemia treatment (claimed) using (A) comprises removing the bone marrow cells, incl. leukaemia cells, from a patient; contacting the cells with M195 or the therapeutic agent and autologously reinfusing the treated cells into the patient. This is carried out in the presence of rabbit, guinea pig or human complement.

Diagnosis of leukaemia is also possible using (A). It is administered to the patient, with an imaging agent attached, to form a complex between (A) and the leukaemic cells, then imaging of the complex and hence diagnosis of leukaemia is carried out.

Genetic information can be carried into a haematopoietic cell (claimed) by contacting the cell with (A) having the information attached. The chimeric Ab can be used instead of (A).

PREFERRED COMPONENTS

The sequence of (A) comprises the hypervariable (HV) region of MAb M195, necessary for antigen binding.

The chimeric Ab comprises 2 dimers each of which has a heavy and a light chain. One chain, at least, comprises

(A) and a constant (C) region. The C region is from a human immunoglobulin (Ig). (A) may further comprise a human framework (FR) region and a C region from a human Ab or from a CDR-related Ab.

The cytotoxic agent is a radio-isotope, e.g. an α-particle emitter selected form Lead-212, Bismuth-212 (pref.) and Astatine-211; or a β-particle emitter selected from Iodine-131 (pref.), Scandium-47, Rhenium-186, Rhenium-188 and Yttrium-90 (pref.). The radio-isotope may also be an auger electron generator selected from Iodine-123 (pref.), Iodine-125, Bromine-77 and Indium-111. It may also be a fissionable nucleotide selected from Boron-10 or an actinide.

The therapeutic agent comprises 50-200 mCi of Iodine-131, 10-50 mCi of Yttrium-90; 20-80 mCi of Bismuth; or 100-300 mCi of Iodine-123.

EXAMPLE

100 µg. of Intact M195 Abs were labelled with 2 mCi Iodine-125 using chloramine-T (2 mg/ml). The Ab was purified using gel exclusion chromatography, then 5 x 10^6 HL60 myeloid leukaemia cells were incubated with 5 µg. of labelled Ab. 200 µl. aliquots of cells were taken at intervals and cell associated radioactivity was determined.

Following centrifugation, internalised radioactivity was determined. Cell surface activity was calculated as being the difference between these two values. Maximal M195 binding was found to be 10,000 sites per cell. Other experiments determined that the amt. of internalised Iodine-125 increased 12-fold following a 12 hr. incubation, compared with time 0.(199pp2041PATDwgNo0/22).
(E) ISR: 8.Jnl.Ref.

91-208094/28 B04 D16 SCHE 20.12.89
SCHERING CORP *WO 9109-059-A
20.12.89-US-453570 (27.06.91) A61k-39/39 C07k-07/08 C07k-15/06 C12p-21/08

New antibody antagonists of human interleukin-4 - used to treat allergies by decreasing IL-4 stimulated mast cell proliferation and IgE prodn.

C91-090256 R(AT BE CH DE DK ES FR GB GR IT LU NL SE OA) N(AU BB BG BR CA FI HU JP KP KR LK MW NO RO SD SU US)

New polypeptides (I) contain 5-26 amino acids and correspond to (part of) the amino acid residues 61-82 or 104-129 of human interleukin-4 (hIL-4).

Also new are
(1) antibody (Ab1) binding specifically to hIL-4 and (I); and
(2) anti-idiotypic antibody (Ab2) against Ab1.

MORE SPECIFICALLY

(I) are of formulae:
Lys-Asp-Thr-Arg-Cys;
Thr-Ala-Gln-Gln-Phe-His-Arg-His;
Lys-Asp-Thr-Arg-Cys-Leu-Gly-Ala-Thr-Ala-Gln-Gln-Phe-His-Arg-His-Lys-Gln-Leu-Ile-Arg -Phe (Ia) or
Ala-Asn-Gln-Ser-Thr-Leu-Glu-Asn-Phe-Leu-Glu-Arg-Leu-Lys-Thr-Ile-Met-Arg-Glu-Lys-Tyr-Ser-Lys-Cys-Ser-Ser.

B(4-B4C5, 4-B4C6, 4-C1, 11-C7A, 12-D2, 12-K4) D(5-C11, 5-H12) B0395

They are pref. coupled to a carrier mol.

USE/ADVANTAGES

Ab1 inhibit binding of IL-4 to cell receptors, by reaction with IL-4, while Ab2 have the same effect by reaction with cellular IL-4 receptors. They are thus useful for treating allergies (by decreasing mast cell growth and IgE prodn.).

The antibodies can also be used to study the mechanism of action of IL-4 and to identify agonists or antagonists.

MATERIALS

(I) are prepd. by standard methods of peptide synthesis or by recombinant DNA technqiues.

Ab1, which may be monoclonal or polyclonal, are made by usual immunisation techniques, opt. using a conjugate of (I) with a carrier (e.g. keyhole limpet haemocyanin) as the immunogen.

Ab2 are prepd. the same way but using Ab1 as the immunogen.

EXAMPLE

Cpd. (Ia), corresp. to amino acids 61-82 of IL-4,

In vitro prodn. of horseshoe crab amoebocytes - useful for Limulus Amoecyte Lysate testing, e.g. of food, surgical instruments, dialysis machines

C90-109896 R(AT BE CH DE ES FR GB IT LU NL SE) N(AU JP KR)

Amoebocyte producing excised Limulus gill flap tissue is used in a method for producing a Limulus lysate (I).

The gill flap tissue is pref. from a horseshoe crab of the following species: Limulus polyphemus, Tachypleus tridentatus, Tachypleus gigas or Carcinoscorpius rotundicauda.

USES /ADVANTAGES

The method provides an in vitro means of producing pyrogen-sensitive (I) in large quantities, which is not dependent on harvesting of wild Limulus polyphemus.

(I) is used as a quantifiable and reliable test for the presence of contamination by Gram-negative bacteria. Limulus amoebocyte Lysate (LAL) testing is used for foods, dialysis machines, surgical instruments and injectable drugs and is exquisitely sensitive to the presence of bacterial endotoxins.

B(4-B2B1, 4-B2B5, 4-B4M, 11-C8E1, 12-K4E) D(5-H4)

5 B0331

DETAILS

The horseshoe crab produces amoebocytes from gill flap tissue, collected in pods, located within the gill flap leaflets.

TISSUE CULTURE

Once isolated, the tissue can be cultured long term in vitro on an artificial surface (pref. collagen, polylysine, fibronectin or polystyrene), or as part of the gill flap leaflets, opened along one edge to allow access of the media to the developing amoebocytes.

Amoebocytes are removed from the gill flaps by pulsing with Limulus serum, copper sulphate or detergent.

EXAMPLE (E) ISR: US4229541 3.Jnl.Ref

After the gill leaflet is opened, both halves are put into a centrifuge tube contg. 20 ml modified Grace's medium. This is laid flat in a shaker with soln. moving the length of the tube.

After 2 - 5 days, 1 - 2 ml of Limulus serum is added. Amoebocytes will release from the leaflet. The emptied leaflet is removed and resuspended into 20 ml modified Grace's. The gill leaflet will become repopulated with amoebocytes. (19ppEDDwgNo0/1). WO 9007860-A

Chimaeric immunoglobulin(s) blocking IL-2 binding to receptors - comprising human framework and murine complementary determining regions, less immunogenic than murine antibodies

C90-109897 R(AT BE CH DE ES FR GB IT LU NL SE OA) N(AT AU BB BG BR CH DE DK FI GB HU JP KP KR LK LU MC MG MW NL NO RO SD SE SU)

A compsn. comprises a pure human-like immunoglobulin (Ig) which (a) reacts specifically with p55 Tac protein and/or (b) inhibits binding of human interleukin-2 (IL-2) to its receptor.

Also new are:
(1) human-like Ig having 2 pairs of light (L)/heavy(H) chain dimers and able to react specifically with an epitope of a human IL-2 receptor with affinity at least 10^8 per mole whereby the chains include CDR's and human-like FR's, both from different Ig molecules;
(2) humanised Ig (hIg) which can bind to IL-2 receptors and contains at least one CDR from anti-Tac antibody (Ab) in a human-like FR contg. at least one amino acid (AA) from the anti-Tac Ab;

B(2-V2, 4-B4A1, 4-B4A3, 4-B4C6, 11-C7A, 12-D2, 12-K4A1) D(5-C12, 5-H7, 5-H8, 5-H12)

7 B0332

(3) nucleic acid (NA) encoding for human-like Fr and at least one murine CDR; and
(4) cells transfected with the NA.

USE/ADVANTAGES

hIG are not significantly immunogenic in humans, are easily and economically produced and have a longer half-life in vivo than mouse Abs. They are useful (opt. when attached to a cytotoxic agent) for treatment of T-cell mediated disorders, e.g. graft or transplant rejection, and autoimmune diseases.

hIG can also be used in vitro for T-cell typing, isolation of IL-2 receptor bearing cells, vaccine prodn., etc.

PROCESS

Four criteria are used in the design of hIG of high affinity for the specific antigen (Ag):

(1) highest possible homology between acceptor (human) and donor (usually mouse) Ig;

(2) if a 'rare' AA is present in the acceptor at a particular position, but a 'common' acid is present at the corresp. donor posn., then the latter is used;

(3) at posns. immediately adjacent to the WO9007861-A+

CDR's, the donor AA is used; and

(4) any AAs outside the CDR's but close enough to interact with it by H-bonding etc., are donor AAs.

A recombinant DNA is constructed from (1) segments encoding L/H chain CDR's (modified if necessary according to the above criteria) capable of specific binding; and (2) segments encoding human FR's. The specification includes the sequence (and derived protein) for the humanised anti-Tac H-and L-chain variable (V) regions. The DNA is placed under control of proper control elements, usually in a vector providing expression in eukaryotic cells.

The DNA can be made by joining genomic sequences or by synthesis.

EXAMPLE

The H-chain of human Ab EU and that of anti-Tac were compared and a chimaeric sequence designed contg. EU AAs except in the CDR's; where EU AAs were 'rare' immediately adjacent the CDR's and where 3-D modelling suggested that an AA was close to the Ag binding site. A similar process was applied to design of chimaeric L-chain.

DNA encoding these designed chains were synthesised, then ligated separately into vectors to give plasmids pHuGTAC and pHuLTAC. These were transfected into mouse Sp2/0 cells and cells which produced hIG were selected. These were injected into mice. the ascites fluid collected and the hIG purified by affinity chromatography.

This humanised Ab was more effective than anti-Tac in causing lysis of the IL-2 receptor bearing human leukaemia cell line HUT-102. (52pp1251PATDwgNo0/10)

(E) ISR: US4816567 EP-239400 WO8901783 GB2188941 7.Jnl.Ref

WO9007861-A